REMARKS

Claims 1, 2, 4-9, and 11-18 are pending after entry of this paper. Claims 1-4, 9-16, and 18 have been rejected. Claims 5-8 and 17 have been withdrawn and claims 3 and 10 have been cancelled without prejudice. Applicants reserve the right to pursue withdrawn and cancelled claims in a divisional or continuing application.

Claim 1 has been amended to incorporate the subject matter of the presently cancelled claims 3 and 10.

No new matter has been introduced by this response. Reconsideration and withdrawal of the pending rejections are respectfully requested.

Response to Rejections under 35 U.S.C. §103

Claims 1-4, 9-16 and 18 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Aznar et al. (*Systematic and Applied Microbiology*, 25:109-119, 2002; of record) in view of Brasher et al. (*Current Microbiology* 37:101-107, 1998; of record). Specifically, the Patent Office alleges that Aznar teaches a method of detecting two or more microorganisms, *i.e.*, different strains of *Listeria monocytogenes*, having different properties as made evident by Table 1 of Aznar, (Office Action; p. 4,) even though, the Patent Office concedes that Aznar does not teach certain limitations such as pH, medium components or chemicals used to lyse the microorganism before DNA extraction. (Office Action; p. 4.) On the other hand, the Patent Office alleges that Brasher teaches a method of detecting *Salmonella*, *Vibrio*, *E. coli* and other bacteria using multiplex PCR amplification of multiple genes. (Office Action; p. 4.) The Patent Office further alleges that Brasher teaches the use of lytic enzyme proteinase K and depositing DNA by alcohol precipitation. The Patent Office concludes that such teachings of

Aznar and Brasher make the claimed invention *prima facie* obvious. The Patent Office alleges that "[o]ne of ordinary skill in the art would have been motivated to by teaching of Aznar et al. to optimize the culture media and pH to obtain the best results for a Multiplex PCR assay. One of ordinary skill in the art would have been also motivated to by teaching of Brasher et al. using a lytic enzyme in the extraction of DNA in the method." (Office Action; p. 4-5.) Applicant respectfully disagree with such reasoning and conclusion arrived by the Patent Office for the following reasons.

1) As an initial matter, in order to expedite prosecution and without disclaimer of, or prejudice to, the subject matter recited therein, applicants have amended claim 1 to incorporate the subject matter of claims 3 and 10. In other words, claim 1 now recites that (1) at least one of the organisms detected is *Listeria monocytogenes* and (2) the microorganisms are cultured in a medium with glucose concentration of 0.15% or less, and/or in a medium with concentration of phosphate-buffer solution of 50 mM or more or in a medium with a buffer ability similar as that with concentration of phosphate-buffer solution of 50 mM or more. Applicants respectfully direct the Patent Office's attention to paragraphs [0025] and [0026], where the influence of buffer ability and sugar concentration of the medium is described:

> As it was thought that the weak proliferation of *Lysteria monocytogenes* in Example 1 was due to the decrease of pH of the medium after culture, the influence of buffer ability and sugar concentration of the medium on the proliferation of each bacterium was investigated. Disodium phosphate and monopotassium phosphate were added to the base medium (tryptose 10 g, meat extract 5 g, yeast extract 5 g, sodium chloride 5 g/1 L) to adjust the phosphate concentration from 15 mM to 200 mM (pH 6.3), and glucose was added by changing the concentration from 0% to 0.25% to prepare the test medium. Meat-derived bacteria and 1 CFU/100 ml each of pathogenic *Escherichia coli* O157, *Salmonella spp.* and *Listeria moncytegenes*, used in Example 1, were inoculated in each test medium where each of these becomes 104 CFU/ml. After culturing at 35°C, counts of general viable

> cells, O157, *Salmonella spp.* and *Listeria*, and the pH levels were measured, 18, 24, 30 and 48 h later. The results are shown in Table 1.
> As a result, by using a medium with glucose concentration of 0.15% or less, or a medium with phosphate concentration of 50 mM or more, or a medium maintaining the pH after culture at 5.1 or more, all of pathogenic *Escherichia coli* O157, *Salmonella spp.* and *Listeria monocytogenes* proliferated to 103 CFU/ml or more (cell counts necessary for detection by PCR) by culturing for 18 h or more. For the tests thereafter, medium No. 17 of Table 1 (tryptose 10 g, meat extract 5 g, yeast extract 5 g, sodium chloride 5 g, glucose 0.5 g, disodium phosphate 7 g, monopotassium phosphate 15 g/1 L) was selected. As for medium components, nitrogen sources other than tryptose, meat extract or yeast extract, carbon sources other than glucose, substances with buffer ability other than phosphate are also effective according to the existing environment or damage level of the bacteria to be detected. Further, it was more preferable to add inorganic salts, pyruvic acid or pyruvate salt, or surfactants such as Tween as substances promoting proliferation.

On the other hand, Aznar describes that a total of 72 strains of *Listeria spp.* were grown in Brain Heart Agar (Merck) (lines 55-56, left column of page 110) and Brasher shows that each strain was cultured on a different medium respectively, stating that *V.parahaemolyticus* was grown on nutrient agar supplemented with NaCl, *V.vulnificus* was cultured on marine agar and that *V.cholerae, S.typhimurium,* and *E.coli* were cultured on LB agar.

Thus, the conditions of the medium employed in the presently claimed method are different from those used by Aznar and Brasher, and the present application describes how two or more microorganisms including *Listeria monocytogenes* having different properties can be effectively grown in one kind of medium, which could not have been expected by a person in the art and would require a great deal of undue experimentation.

2) Furthermore, "[i]n determining the differences between the prior art and the claims, the question under 35 U.S.C. 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious."

Stratoflex, Inc. v. Aeroquip Corp., 713 F.2d 1530, 218 USPQ 871 (Fed. Cir. 1983) (cited in MPEP 2141.02). The methods described in Aznar and Brasher are designed for different bacterial species and those skilled in the art would not look to one to compensate for the shortcomings of the other. For instance, although Brasher describes the detection of two or more microorganisms with different properties, i.e., *E.coli*, *Salmonella* and *Vibrio* spp, it is important to note that all of these species belong to a family of Gram negative bacteria. On the other hand, Aznar describes the detection of multiple *Listeria monocytogenes* strains, which are a species of a Gram positive bacteria (*i.e.*, a thicker and higher density peptide glycan layer) belonging to the *Listeria* family. It is relatively simpler to proliferate bacteria simultaneously when the target microorganisms belongs to the same family or bacterial species of same genus, but it is difficult and not obvious to do so for microorganisms of diverse types such as *E. coli*, *Salmonella spp.* and *Listeria monocytogenes*. For example, *Listeria* grows at a low temperature and proliferates slower compared to *Salmonella spp.* and *E. coli O157*. Thus, the DNA extraction method(s) that work for the species that have a Gram-negative cell wall do not necessarily mean that the same extraction method(s) would work for species that have a Gram-positive cell wall and vice versa. A skilled artisan would have to conduct a great deal of undue experimentation in order to arrive at the claimed invention by combining two different inventions into one as suggested by the Patent Office based on the species-specific teachings of Aznar and Brasher. At least on this ground, the combination of Aznar and Brasher does not make the claimed invention *prima facie* obvious.

3) Finally, assuming for an argument sake that those skilled in the relevant art could combine Aznar with Brasher, applicants still respectfully assert that if such combination "would render the prior art invention being modified unsatisfactory for its intended purpose, then

there is no suggestion or motivation to make the proposed modification." *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984) (cited in 2143.01). Aznar employs the Pitcher method of extracting the DNA from *Listeria moncytogenes* with guanidium thiocyanate. According to the Patent Office, a skilled artisan would be motivated to use "lytic enzyme in the extraction of DNA in the method." (Office Action; p. 5.) Although, Brasher describes the use of lytic enzyme, i.e., proteinase K, it is improper to consider such teaching without considering Basher "as a whole." "Distilling an invention down to the 'gist' or 'thrust' of an invention disregards the requirement of analyzing the subject matter 'as a whole.'" *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983). Specifically, in addition to the use of proteinase K, Brasher also teaches the use of SDS and organic solvents such as chloroform and phenol in the DNA extraction. While SDS is known as a surfactant that is easy to use in DNA extraction, it is a strong PCR inhibitor and must be removed completely after being used for extraction. Furthermore, a small contamination with SDS may also cause significant fluctuations in extraction efficiency between samples, and, therefore, very much undesirable.

Additionally, the Patent Office states that Brasher teaches that DNA was precipitated by alcohol citing page 102 of Brasher (Office Action, p. 4). However, to be more specific, Brasher describes that DNA was purified with chloroform-isoamyl alcohol followed by centrifugation and that further purification of the DNA in the supernatant was achieved by extracting with phenol-chloroform-isoamyl alcohol. Phenol and chloroform are dangerous and harmful organic solvent that are not suitable for examination of pathogens in food manufacturing sites. (Specification as filed; para. [0009] page 7).

On the other hand, it is readily noted in the instant specification that "even phenol or chloroform treatment is not performed, protein being soluble to a level sufficient to be

detected by PCR without problem can be removed by alcohol precipitation or according to the added level of DNA extraction solution (2 µl per 50 µl of PCR reaction solution) "(paragraph [0011]). Thus, Brasher and the instant invention are quite different in the effect and the utilization of a Brasher method would make the Aznar method unsatisfactory for its intended purpose of testing *Listeria moncytogenes* in food and/or food manufacturing sites.

In view of the aforementioned remarks and claim amendments, applicants respectfully asset that the instant invention is not made obvious by Aznar in view of Brasher. Therefore, applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. §103(a) rejection of claims 1-4, 9-16 and 18 as being obvious over Aznar in view of Brasher.

Dependent Claims

The applicants have not independently addressed all of the rejections of the dependent claims. The applicants submit that for at least similar reasons as to why independent claim(s) 1 from which all of the dependent claims 2, 4, 9, 11-16 and 18 depend are believed allowable as discussed *supra*, the dependent claims are also allowable. The applicants however, reserve the right to address any individual rejections of the dependent claims and present independent bases for allowance for the dependent claims should such be necessary or appropriate.

CONCLUSION

Based on the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the restriction requirement imposed on the pending claims and allowance of this application. Favorable action by the Examiner is earnestly solicited.

AUTHORIZATION

The Commissioner is hereby authorized to charge any additional fees which may be required for consideration of this Amendment to Deposit Account No. **50-4827**, Order No. 1004451.001US.

In the event that an extension of time is required, or which may be required in addition to that requested in a petition for an extension of time, the Commissioner is requested to grant a petition for that extension of time which is required to make this response timely and is hereby authorized to charge any fee for such an extension of time or credit any overpayment for an extension of time to Deposit Account No. **50-4827**, Order No. 1004451.001US.

Respectfully submitted,
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